

Claims:

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1. A method of modifying a nucleic acid molecule comprising;
contacting the nucleic acid molecule with a prokaryotic DNA
repair ligase polypeptide.

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2. A method according to claim 1 wherein the prokaryotic DNA repair
ligase polypeptide comprises one or more of: a primase domain, a
nuclease domain, and a ligase domain, said one or more domains
sharing greater than 20% sequence identity with the corresponding
domain sequence of Mt-Lig (CAB08492).

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3. A method according to claim 1 or claim 2 wherein the prokaryotic
DNA repair ligase polypeptide shares greater than 20% sequence
identity with the sequence of Mt-Lig (CAB08492).

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4. A method according to any one of claims 1 to 3 wherein the
prokaryotic DNA repair ligase polypeptide is Mt-Lig (CAB08492) or a
variant or allele thereof.

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5. A method according to any one of the preceding claims wherein
the nucleic acid molecule and the Mt-Lig polypeptide are contacted
in the presence of a prokaryotic Ku polypeptide.

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6. A method according to claim 5 wherein the prokaryotic Ku
polypeptide shares greater than 20% sequence identity with the
sequence of Mt-Ku (CAB08491).

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7. A method according to claim 6 wherein the prokaryotic Ku
polypeptide is Mt-Ku (CAB08491) or an allele or variant thereof.

8. A method of ligating nucleic acid molecule ends comprising;
contacting a first nucleic acid end and a second nucleic acid
end with an prokaryotic DNA repair ligase polypeptide,

wherein said first and said second nucleic acid ends are non-compatible.

9. A method according to claim 8 wherein said first and said
5 second nucleic acid ends comprise non-complementary overhang
regions.
10. A method according to claim 8 or claim 9 wherein the first end
10 is on a first nucleic acid molecule and the second end is on a
second nucleic acid molecule.
11. A method according to claim 10 wherein the first and second
nucleic acid molecules are DNA.
- 15 12. A method according to claim 10 wherein the first nucleic acid
molecule is DNA and the second nucleic acid molecule is RNA.
13. A method according to claim 8 or claim 9 wherein the first and
second ends are on the same nucleic acid molecule.
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14. A method according to any one of claims 8 to 13 comprising
isolating and/or purifying the ligated nucleic acid molecule.
15. A method of labelling a nucleic acid molecule comprising;
25 contacting a nucleic molecule having a first terminus with an
prokaryotic DNA repair ligase polypeptide in the presence of
labelled nucleotides.
16. A method according to claim 15 wherein the nucleotides are
30 NTPs.
17. A method according to claim 15 wherein the nucleotides are
dNTPs.
- 35 18. A method of filling in a single stranded gap in a double
stranded nucleic acid molecule comprising;

contacting a double stranded nucleic acid molecule having a single stranded region with an prokaryotic DNA repair ligase polypeptide.

5 19. A method according to claim 18 wherein said nucleic acid molecule and said prokaryotic DNA repair ligase polypeptide are contacted in the presence of NTPs.

10 20. A method according to claim 18 wherein said nucleic acid molecule and said prokaryotic DNA repair ligase polypeptide are contacted in the presence of dNTPs.

21. A method of removing a single stranded overhang from the end of a nucleic acid molecule comprising;
15 contacting said nucleic acid molecule with a prokaryotic DNA repair ligase polypeptide

22. A method according to claim 21 wherein the prokaryotic DNA repair ligase polypeptide is an Mt-Lig polypeptide.

20 23. A method according to claim 21 or claim 22 wherein said nucleic acid molecule is contacted in the presence of Mg^{2+} or Mn^{2+} .

24. A method of producing an RNA molecule comprising;
25 contacting a prokaryotic DNA repair ligase polypeptide and a template DNA strand in the presence of NTPs.

25. A method according to claim 24 wherein prokaryotic DNA repair ligase and template DNA are contacted in the presence of a primer
30 oligonucleotide.

26. A method of producing an DNA molecule comprising;
contacting A prokaryotic DNA repair ligase polypeptide and a nucleic acid template in the presence of dNTPs and a primer oligonucleotide.

35 27. A method according to claim 26 wherein the nucleic acid template is an RNA template.

29. A method according to any one of claims 8 to 28 wherein the prokaryotic DNA repair ligase polypeptide comprises one or more of: a primase domain, a nuclease domain, and a ligase domain, said one or more domains sharing greater than 20% sequence identity with the corresponding domain sequence of Mt-Lig (CAB08492).

30. A method according to any one of claims 8 to 29 wherein the prokaryotic DNA repair ligase polypeptide shares greater than 20% sequence identity with the sequence of Mt-Lig (CAB08492).

31. A method according to any one of claims 8 to 30 wherein the prokaryotic DNA repair ligase polypeptide is Mt-Lig (CAB08492) or a variant or allele thereof.

32. A method according to any one of claims 8 to 31 wherein the nucleic acid molecule and the Mt-Lig polypeptide are contacted in the presence of a prokaryotic Ku polypeptide.

33. A method according to claim 32 wherein the prokaryotic Ku polypeptide shares greater than 20% sequence identity with the sequence of Mt-Ku (CAB08491).

34. A method according to claim 32 or claim 33 wherein the prokaryotic Ku polypeptide is Mt-Ku (CAB08491) or an allele or variant thereof.

35. A kit comprising an isolated Mt-Lig polypeptide for use in a method according to any one of claims 1 to 34.

36. A kit according to claim 35 comprising an isolated Mt-Ku polypeptide.

37. A kit according to claim 35 or claim 36 comprising dNTPs.

38. A kit according to claim 35 or claim 36 comprising NTPs.

39. A kit according to any one of claims 35 to 38 comprising one or more of buffers, stabilisers and excipients.

40. A method of producing an prokaryotic DNA repair polypeptide

5 comprising;

(a) causing expression from nucleic acid which encodes a prokaryotic DNA repair polypeptide in a suitable expression system to produce the polypeptide recombinantly; and, testing the recombinantly produced polypeptide for prokaryotic DNA
10 repair activity.

41. A method according to claim 40 wherein the recombinantly produced polypeptide is tested for one or more of: non-complementary end ligation activity, DNA dependent RNA primase
15 activity, 3'-5' exonuclease activity, DNA and RNA dependent DNA polymerase activity, DNA dependent RNA polymerase activity, ATP dependent DNA and RNA ligase activity and DNA terminal transferase activity.

20 42. A method according to claim 39 or 40 wherein the prokaryotic DNA repair polypeptide is an Mt-Lig polypeptide or an allele or variant thereof.

43. A method according to any one of claims 39 to 41 comprising
25 purifying said recombinantly produced polypeptide.